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The Fine Mapping and Characterization of the Barley Desynaptic Mutant *des12*

Sybille Mittmann[1,2], Luke Ramsay[1], Malcolm Macaulay[1], Robbie Waugh[1,2], Isabelle Colas[1]
[1]The James Hutton Institute, Invergowrie, Dundee DD2 5DA, Scotland
[2]University of Dundee, College of Life Sciences – Division of Plant Science, Invergowrie, Dundee DD2 5DA, Scotland, UK
Email: sybille.mittmann@hutton.ac.uk

Introduction

In the cereals such as barley and wheat, recombination is skewed towards the telomeric regions of the chromosomes. This results in the finding that large portions of the cereal genomes hardly ever recombine. This negatively impacts on breeding schemes that want to create new allele combinations and replace disadvantageous alleles. Studies are under way to improve recombination in the centromeric regions. At the James Hutton Institute we characterize a collection of barley desynaptic mutants to discover novel genes controlling meiosis with the ultimate aim of being able to modify the pattern of recombination. The following study used a forward genetics approach by fine mapping and cytologically characterizing the desynaptic mutant *des12*. *des12* is a spontaneous mutation, which was found in the cultivar Betzes and has two alleles. Near-isogenic lines (BW233 and BW232) were created by backcrossing the two alleles with the common cultivar Bowman (wt). In the following a segregating F_2 BW233xMorex population was initially mapped using the 384 BeadXpress genotyping platform; mapping *des12* to the long arm of 7H. Phenotypically *des12* is severely semi-sterile (Figure 1) and is characterized by an abnormal synaptonemal complex during prophase I leading to improper chromosome segregation due to missing 'linking' chiasmata. The interest was to map and identify the underlying gene as well as to characterize its function during meiosis.

Results

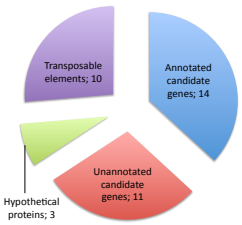

Diagram 1: The *des12* interval contains 38 gene models in rice of which only a portion are regarded as candidate genes, the rest are either transposable elements that are specific to rice or are hypothetical proteins.

The previously 17.2 cM large interval was narrowed down to roughly 1 cM, containing 38 gene models in rice of which about 25 gene models are potential candidate genes (Diagram 1). No obvious meiotic proteins were found in this region, but two genes showed promising transcriptome expression profiles in rice (Rice Genome Annotation Project). The first one is a potential RING-type E3 ubiquitin ligase that is involved in post-transcriptional regulatory processes, and the other is a ligaseA, which ligates DNA inter alia during DNA replication and repair. Both gene families have been shown to regulate or act in meiosis.
Initial cytological analysis (Figure 4) using a DNA stain and antibodies against the meiotic protein ASY1, which visualizes the homologous chromosomes, revealed a potentially new characteristic; an arrest at diplotene, which remains to be confirmed in a more thorough investigation.

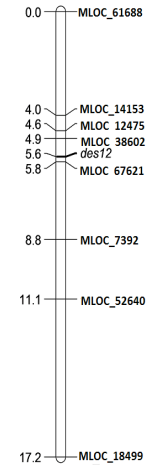

Figure 3: Calculated linkage map of *des12* interval on the long arm of chromosome 7H using JoinMap 4.1 , (Stam 1995). The mapping positions are based on genotyping data of the 937 individuals of the F_2 BW233xBarke population with the markers shown in conjunction with the phenotypic data.

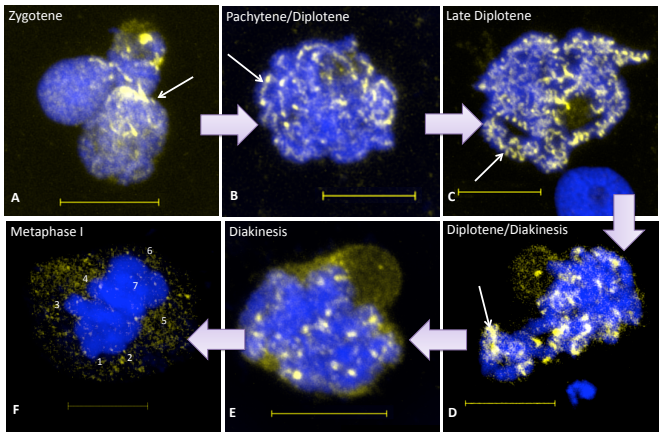

Figure 4: Confocal images of the immunocolocalization Atlas of *des12*, plant 2-5 (assumed mutant). The images show the progression of meiosis I. Blue colour: DNA stain HOECHST 33342. Yellow colour represents the axial elements of the chromosomes visualized by anti-ASY1 antibodies paired with alexa fluor 561. The scale bar indicates 10 µm. A At early zygotene the bouquet forms, which is made up of the telomeres of the chromosomes attaching to the same place on the nuclear envelope (arrow). B At pachytene the homologous chromosomes have paired (see arrow). At diplotene the chromosomes unpair and the chromatin becomes loose. C At late diplotene in *des12* spiralling and coiling of the chromatin can be observed (arrow). D In the transition of diplotene to diakinesis the chromosomes condense further and the bivalents become visible (arrow). E At diakinesis the bivalents are visible. F At metaphase I the clearly visible ring bivalents (see numbers) align on the metaphase plate.

Methods

960 plants of the F2 BW233xBarke population were grown and phenotyped for sterility/fertility to delineate the *des12* interval further using KASPar assays and sequencing. Primers were developed using existing barley genomic data as well as synteny comparison with rice and *Brachypodium* (Figure 2).

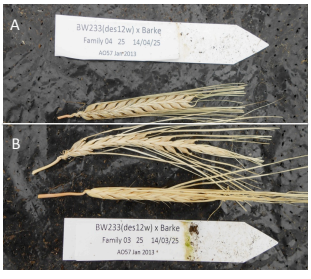

Figure 1: Comparison of wildtype (WT) and *des12* phenotype. (A) Plant 14/04/25 from the cross BW233xBarke was genotyped as Barke allele, which was confirmed by its fertile phenotype (on top). (B) Plant 14/03/25 is semi-sterile to sterile and contains the *des12* allele.

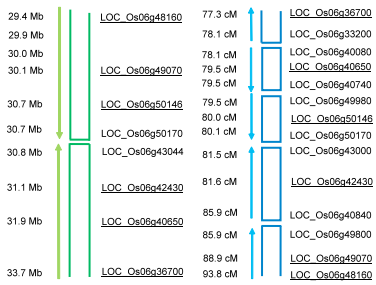

Figure 2: Breakpoint cartoon of *des12* region in *Brachypodium* and barley described in rice orthologues. Underlined rice orthologues highlight the location of the SNPs used.

Conclusions

- Using the newly available high density and integrated barley genetic map as well as rice annotation, it is now possible to map traits or delineate regions with much more ease and speed than ever before
- On-going marker/primer development to further delineate *des12* interval and sequence promising candidate genes
- Cytological analysis of *des12* to characterize its function in meiosis and recombination (Figure 4 - immunocolocalization)